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PATENT
Attorney Docket No.: 020130-001420US

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

On 1 Dec. 2006
TO TOWNSEND and TOWNSEND and CREW LLP

Malinda C. Asif

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

VANDER HORN, Peter B.

Application No.: 10/627,592

Filed: July 25, 2003

For: METHODS OF MAKING HYBRID
PROTEINS

Customer No.: 20350

Confirmation No. 2975

Examiner: Jeffrey S. Lundgren

Technology Center/Art Unit: 1639

DECLARATION UNDER 37 C.F.R. §
1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Peter B. Vander Horn hereby declare that:

1. I am the inventor of the subject matter claimed in the above-referenced patent application.
2. I conceived of and reduced to practice the claimed invention in the United States prior to November 2002. Attached Exhibits A and B provide evidence of the completion of the invention. The dates on the Exhibits and unrelated information have been redacted. All redacted dates are prior to November 2002.
3. The present invention relates, in part, to methods of generating hybrid proteins. In the methods of the invention, at least two parent protein sequences that have a common biological activity are aligned and positions at which the parent amino acid residues are different are identified (such a position is referred to herein as a "divergent site"). The codons

that encode the differing residues are compared and a nucleic acid sequence encoding a protein that is a hybrid of the parent proteins is derived. This nucleic acid sequence includes degeneracies at codons that encode divergent sites. Such a degenerate codon has at least one nucleotide position in the codon that is variable such that the codon can encode multiple parental amino acid residues at the divergent site, depending on which nucleotide is incorporated at that codon position during synthesis of a nucleic acid molecule. Libraries can thus be generated in which members are hybrids that have amino acid residues from one of the parents at some of the divergent sites and, independently, amino acid residues from a different parent at other divergent sites. The library is then screened and functional hybrid proteins are identified.

4. Prior to November 2002, I aligned a parent Pfu polymerase protein sequence and a parent Deep Vent® polymerase protein sequence and identified differences in the amino acid sequences. An *E. coli* codon usage table was used to compare the various codons that can encode the differing amino acids. I created a nucleic acid sequence that alternatively encoded differing parental amino acid residues at sites of variation in the protein sequences.

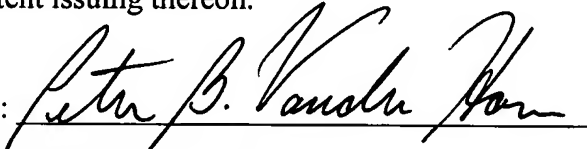
5. Oligonucleotides were designed for synthesis to assemble together to form a full length polymerase gene to make a library encoding hybrid polymerase proteins. A copy of a laboratory notebook page showing the sequences of the oligonucleotides, including the positions that can encode alternative amino acid residues at sites that differ in the parent protein sequences, is provided in Exhibit A. The oligonucleotides were synthesized and assembled by overlap extension.

6. Functional polymerase proteins encoded by members of the library were identified. An example of a PCR analysis using an exemplary hybrid polymerase protein that was isolated from the library is shown in the copy of a laboratory notebook page provided as Exhibit B. Exhibit B shows a gel with the products of amplification reactions performed using a hybrid polymerase, designated "PhS1". The hybrid polymerase amplified template DNA targets of 4, 5, 9, and 13 kb in length. The gel was obtained prior to November 2002.

7. In view of the foregoing, I respectfully submit that it has been unequivocally established that the claimed invention was conceived of and reduced to practice prior to November 2002.

I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed:


Peter B. Vander Horn

Dated:

10/30/2006

Attachments: Exhibits A and B

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* probably due to slightly higher effective [free Hg^{2+}] (i.e. pH of 6.5 vs 6.0)

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